Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2023 January 10.

Published in final edited form as:

Stem Cell Res. 2022 December ; 65: 102958. doi:10.1016/j.scr.2022.102958.

Generation and characterization of a human induced pluripotent stem cell (iPSC) line from a patient with congenital heart disease (CHD)

Hui Lin[a], **Shi-Qiao Ye**[a], **Zhao-Hui Xu**[b,c], **Jacqueline S. Penaloza**[d], **Mona Aljuhani**[a], **Tatyana Vetter**[e], **Ming-Tao Zhao**[a,c,*], **Kim L. McBride**[a,c,*]

[a]Center for Cardiovascular Research and The Heart Center, Nationwide Children's Hospital, Columbus, OH, USA

[b]Center for Vaccines and Immunity, Nationwide Children's Hospital, Columbus, OH, USA

[c]Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH, USA

[d]Computational Genomics Group, The Steve and Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH, USA

[e]Center for Gene Therapy, Nationwide Children's Hospital, Columbus, OH, USA

Abstract

Epstein-Barr virus (EBV) immortalized lymphoblastoid cell lines (LCLs) are widely used for banking. This bioresource could be leveraged for creating human iPSC lines to model diseases including CHD. We generated an LCL-derived iPSC line (NCHi001-A) from a patient with congenital aortic valve stenosis. NCHi001-A was EBV and transgenes free, exhibited stem cell-like morphology, expressed pluripotency markers, has a normal karyotype, and could be differentiated into cells of three germ layers in vitro. Relationship inference via a microarray-based analysis showed NCHi001-A is identical to the parental cell line. NCHi001-A can be used for disease modeling, drug discovery, and cell therapy development.

1. Resource Table

Unique stem cell line identifier	**NCHi001-A**
Alternative name(s) of stem cell line	LCL-iPSC4802
Institution	Nationwide Children's Hospital

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

*Corresponding authors at: Center for Cardiovascular Research and The Heart Center, Nationwide Children's Hospital, Columbus, OH, USA. Kim.mcbride@nationwidechildrens.org (K.L. McBride).

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102958.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Unique stem cell line identifier	NCHi001-A
Contact information of distributor	Kim.mcbride@nationwidechildrens.org
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age:31-year-old Sex: Female Ethnicity if known: Caucasian
Cell Source	EBV immortalized human lymphoblastoid cell line (LCL)
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	NO
Type of Genetic Modification	NO
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Congenital aortic valve stenosis
Gene/locus	A homozygous *NFATC1* variant; chr18:77,153,996 G > A (GRCh37/hg19)
Date archived/stock date	9th March 2022
Cell line repository/bank Ethical approval	https://hpscreg.eu/cell-line/NCHi001-A Genetics of CVD IRB09-00339

2. Resource utility

This iPSC line from a patient with congenital aortic valve stenosis containing the homozygous NFATC1 enhancer variant 18:77,153,996 G > A provides an in vitro cellular model to study the role of NFATC1 in the pathogenesis of CHD and potentially facilitates the development of novel therapeutic interventions. Table 1..

3. Resource details

Congenital heart disease (CHD) is a common birth defect, affecting 6 to 8 per 1000 live births. Human induced pluripotent stem cells (iPSCs) have opened new approaches to investigate genetic mechanisms of CHD using clinically relevant and patient-specific cardiac cells (e.g., cardiomyocytes, endothelial/endocardial cells) (Lin, McBride et al. 2021).

Human iPSCs have been derived from a variety of cell sources, including EBV-immortalized lymphoblastoid cells lines (LCLs), which are widely used for cell line banking. Bio-banked LCLs are an underutilized resource for generating iPSCs useful for elucidating disease mechanisms of CHD. Here, we generated an iPSC line NCHi001-A, from an EBV-immortalized LCL derived from a 31-year-old female diagnosed with congenital aortic valve stenosis, using the CytoTune™-iPSC 2.0 Sendai Reprogramming kit (Thermo Fisher Scientific).

We reprogrammed LCL4802 with Sendai virus (SeV) containing 4 Yamanaka classical factors: OCT4, KLF4, C-MYC and SOX2 (Zhao et al., 2017; Martineau, Racine et al. 2018). On day 15, iPSCs clones had stem cell-like morphologies with a high nuclear-to-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cytoplasmic ratio, and individual colonies were picked and expanded in culture (Fig. 1A). At passage 10, PCR detected no EBV-related genes in the iPSC line NCHi001-A or negative control (NTC), which were present in the parental LCL4802 control (Fig. 1B). In addition, reprogramming transgenes including SeV and KOS were not detected in genomic DNA of NCHi001-A by RT-PCR analysis (Fig. 1D). Immunofluorescent staining was positive for the pluripotent surface antigen TRA-1–60 (99 %) and nuclear pluripotency markers OCT4A (99 %), C-MYC (99 %), NANOG (95 %) and SOX2 (94 %) (Fig. 1E). To evaluate tri-lineage differentiation, iPSCs were differentiated into three germ layers using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems). We confirmed the expression of ectoderm (Otx2/SOX1), mesoderm (Brachyury/HAND1), and endoderm (SOX17/GATA-4) markers by immunofluorescence staining (Fig. 1F). These results indicate that these iPSCs have ability to differentiate into cells from all 3 germ layers. We confirmed that NCHi001-A was identical to the parental LCL using KING software (Kinship = 0.5). The karyotype of these iPSCs was normal, and there was no detectable gain or lost (greater than5MB) in all chromosomes (Fig. 1C and Supplementary Fig. 1).

4. Materials and methods

4.1. Cell culture

LCLs isolated from blood were transfected using EBV into immortalized cells using an established protocol (Gilbert and Haines, 2001). EBV-immortalized LCLs were maintained with RPMI1640 (Gibco) containing 20 % FBS at 37 °C, 5 % CO_2, and atmospheric O_2.

4.2. Reprogramming

EBV-immortalized LCLs were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). On day 0, 100,000 were transfected using Sendai virus. On day 1, cells were reseeded into another well of a 24-well plate. On day 3, cells were transferred into a Matrigel coated 24-well plate pre-loaded with Repro-TeSR™ medium. Medium was refreshed every day. On day 12 to 15, when iPSC clones appeared, mTesR™1 medium was started. After transduction by day 18 to 21, colonies were individually picked and transferred to a new well for expansion. All experiments were performed using iPSCs at least passage 10.

4.3. Rt-pcr

Total RNA was extracted from iPSCs after passage 10 using RNeasy mini-Kit (QIAGEN). Reverse transcription was carried out using Verso cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer’s instruction (Table 2).

4.4. Immunofluorescence staining

Cells were seeded on 12 mm coverslips coated with Matrigel in 24-well plate. Cells were rinsed twice with DPBS, then fixed using 0.5 mL of 4 % PFA for 10 min at room temperature. PFA was aspirated, and cells were washed with DPBS for 5 min, then gently rinsed with 0.2 M glycine in PBS for 10 min on a shaker. If permeabilization was needed, cells were incubated with 0.5 Triton X-100 plus 1 % protease inhibitor in DPBS for 10 min. Samples were washed three times with DPBS for 10 min on a shaker. For blocking, cells

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

were incubated with 10 % serum from the source species of the secondary antibody raised for 1 h at room temperature. Cells were incubated in 250 μL of primary antibody (Table 2) diluted in blocking buffer in dark overnight at 4 °C. Samples were washed three times with the blocking buffer each for 10 min with shaking. Cells were incubated with the secondary antibody diluted in the blocking buffer for 1 h, unless the primary antibody was conjugated to a fluorochrome. Samples were washed three times with DPBS each for 10 min with shaking. Coverslips were mounted with Invitrogen ™ ProLong ™ Gold Antifade Mountant with DAPI.

4.5. Microscopy

Images were captured using a motorized Nikon Eclipse Ti2-E microscope with a 40X Plan Apo Lambda objective, a Lumencor SOLA LED engine, and a Hamamatsu ORCA Fusion camera.

4.6. Tri-lineage differentiation

The Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) was used to functionally test the ability of iPSCs to differentiate into cells of three germ layers.

4.7. Karyotyping

Genomic DNA samples were sent to Roswell Park Comprehensive Cancer Center for Illumina Infinium CytoSNP-850 k array analysis. The bioinformatic analysis was performed by using KaryoploteR (version 1.20.0), a Bioconductor package. This R package was used to visualize and identify the distributions of existing variations in the genome on interest.

4.8. Mycoplasma detection

Mycoplasma contamination was evaluated using Venor™GeM Mycoplasma Detection Kit, PCR-based (Sigma-Aldrich) following the manufacturer's instructions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by Nationwide Children's Hospital Foundation. Kim L McBride is supported by Nationwide Children's Hospital Foundation, Additional Ventures Innovation Fund and NHLBI R01HL109758. This work was partially supported by the American Heart Association (AHA) Career Development Award 18CDA34110293 (M-T.Z.), NIH/NHLBI R01HL155282 (M-T.Z.), R21HL165406 (M-T.Z.), Additional Ventures Innovation Fund (AVIF) and Single Ventricle Research Fund (SVRF) (M-T.Z.).

Data availability

No data was used for the research described in the article.

Abbreviations:

EBV	Epstein Barr virus

Author Manuscript

LCL	lymphoblastoid cell line

References

Gilbert J (2001). "Establishment of permanent cell lines by Epstein-Barr virus transformation." Current protocols in human genetics / editorial board, Haines Jonathan L. … [et al.] Appendix 3: Appendix3H.

Lin H, McBride KL, Garg V, Zhao MT, 2021. Decoding Genetics of Congenital Heart Disease Using Patient-Derived Induced Pluripotent Stem Cells (iPSCs). Front. Cell Dev. Biol 9.

Martineau L, et al. , 2018. Lymphoblastoids cell lines - Derived iPSC line from a 26-year-old myotonic dystrophy type 1 patient carrying (CTG)200 expansion in the DMPK gene: CHUQi001-A. Stem Cell Res. 26, 103–106. [PubMed: 29274549]

Zhao MT, et al. (2017). "Molecular and functional resemblance of differentiated cells derived from isogenic human iPSCs and SCNT-derived ESCs." Proceedings of the National Academy of Sciences 114(52): E11111–E11120.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

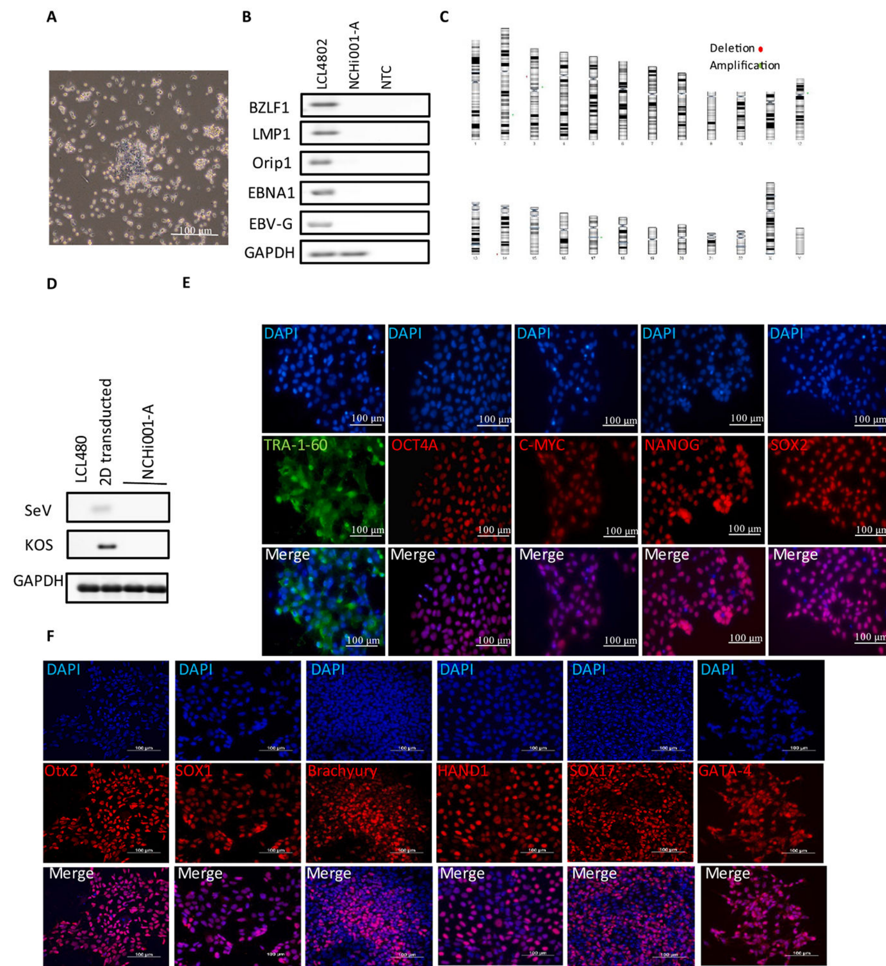

Fig. 1.

Characterization of NCHi001-A iPSC line.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis	Expression of pluripotency markers: TRA 1–60, OCT4A, C-MYC, NANOG and SOX2	Fig. 1E
	Quantitative analysis	Pluripotent markers positive cells: TRA 1–60: 99 %; OCT4A: 99 %; C-MYC: 99 %; NANOG: 95 % and SOX2: 94 %	Fig. 1E
Genotype	Illumina Infinium CytoSNP-850 K array analysis	Karyotype based microarray analysis confirmed the gain or loss of any significant no more than 5 MB	Fig. 1C
Identity	Illumina Infinium CytoSNP-850 K array analysis	Karyotype is normal	Supplementary Fig. 1
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, Negative	Supplementary Fig. 2
Differentiation potential	Directed differentiation	Positive expression of several lineage specific genes markers assessed with immunocytochemistry.	Fig. 1F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: Otx2 and SOX1, Mesoderm: Brachyury and HAND1 Endoderm SOX17 and GATA-4	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-TRA-1–60	1:100	Invitrogen Cat # 41–1000	RRID: AB_2533494
	Rabbit anti-Oct-4A	1:400	Cell Signaling Cat# 2840P	RRID: AB_2167691
	Rabbit anti-C-MYC	1:800	Cell Signaling Cat# 5605P	RRID: AB_1903938
	Rabbit anti-Nanog	1:200	Cell Signaling Cat# 4903P	RRID: AB_10559205
	Rabbit anti-Sox2	1:400	Cell Signaling Cat# 3579P	RRID: AB_2195767
Differentiation Markers	Goat anti-Otx2	10 ug/mL	R&D SYSTEMS Cat# AF1979	RRID: AB_2157172
	Goat anti-SOX1	10 ug/mL	R&D SYSTEMS Cat# AF3369-SP	RRID: AB_2239879
	Goat anti-Brachyury	10 ug/mL	R&D SYSTEMS Cat# AF2085	RRID: AB_2200235
	Goat anti-HAND1	10 ug/mL	R&D SYSTEMS Cat# AF3168-SP	RRID: AB_2115853
	Goat anti-SOX17	10 ug/mL	R&D SYSTEMS Cat# AF1924	RRID: AB_355060
	Mouse anti-GATA-4	15 ug/mL	R&D SYSTEMS Cat# MAB26061-SP	RRID: Not in database
Secondary antibodies	Rabbit anti-Sox2Alexa Fluor 488 Goat anti Mouse IgG (H + L)	1:2000	Invitrogen Cat# A11001	RRID: AB_2534069
	Alexa Fluor 488Goat anti Rabbit IgG (H + L) Alexa Fluor 594	1:1000	Invitrogen Cat# A11012	RRID: AB_2534079
	Alexa Fluor 594			
	Donkey anti-Mouse IgG NorthernLights™ NL557-conjugate Antibody	1:200	R&D SYSTEMS Cat# NL007	RRID: AB_663768
	Donkey anti-Goat IgG NorthernLights™ NL557-conjugated Antibody	1:200	R&D SYSTEMS Cat# NL001	RRID: AB_663766
Primers	**Target**	**Size of band**	**Forward/reverse primer (5′-3′)**	
EBV related genes (PCR)	BZLF-1	638 bp	TGAAGCAGGCGTGGTTTCAA	
		617 bp	CACCTCAACCTGGAGACAAT	
	LMP1	545 bp	TGAGCAGGATGAGGTCTAGG	
		181 bp	ATGGAACACGACCTTGAGA	
SeV genome and transgene (RT-PCR)	OriP	528 bp	TCGGGGGTGTTAGAGACAAC	
		556 bp	TTCCACGAGGGTAGTGAACC	
	SeV		GGA TCA CTA GGT GAT ATC GAG C	
House-Keeping Gene (RT-PCR)			ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	
	KOS		ATG CAC CGC TAC GAC GTG AGC GC	
			ACC TTG ACA ATC CTG ATG TGG	

GAPDH	ACCACAGTCCATGCCATCAC TCCACCACCCTGTTGCTGTA

Author Manuscript Author Manuscript Author Manuscript Author Manuscript